

complexes, the structure was determined with the isolated RBD, without the catalytic portions of the effector molecules. The PI3K γ structure shows how the RBD interacts with the remainder of the enzyme. The RBD of PI3K γ contacts the N-lobe and to a lesser degree the C-lobe of the catalytic domain. RBD residues in R α 2 and the R β 3/R β 4 loop interact with the catalytic domain, mainly with the k β 1/k β 2 and k β 4/k β 5 loops and helix k α 6. The position of the RBD of PI3K γ in relationship to the remainder of the enzyme allows for two possible mechanisms by which Ras binding might cause effector activation. One possibility is a recruitment mechanism whereby Ras increases PI3K activity by translocating the enzyme to the plasma membrane. A second possibility would be an allosteric mechanism in which Ras binding to the RBD causes a conformational change that would be propagated through the RBD/catalytic domain interface to affect substrate or co-factor binding.

By superimposing the RBDs of RalGDS and PI3K γ , it is possible to construct a model of Ras interaction with PI3K γ (Fig. 5). With this model, we can rationalise the differential effects of various switch I and switch II mutants on PI3K binding as opposed to other effectors. Mutations in Ras switch I residues T35S and D38E eliminate PI3K binding, but do not affect Raf binding (15). The E37G mutation abolishes binding to PI3K and Raf but not to RalGDS. The Y40C mutation does not affect PI3K binding, but abrogates Raf and RalGDS binding. In the switch II region, the Y64G mutation eliminates PI3K and neurofibromin binding but has no effect on Raf binding (14). In the model of the PI3K/Ras interaction, residues E37, D38, Y40 and Y64 would be at the PI3K/Ras interface. PI3K K234 would be in a position to form a salt bridge to E37 of Ras and K255 at the C-terminal end of R α 1 could form a salt link with D38 of Ras. K255 in PI3K γ is probably analogous to K227 in PI3K α .

Mutation K227E blocks PI3K α binding to Ras (15). Y40 interacts with K32 in RalGDS (numbering as in (17)). However, because of a very different orientation of the K32 equivalent in PI3K γ (K234) this interaction may not be possible. This could account for the insensitivity of PI3K to the Y40C mutation. On the other hand, Y64 in switch II would be in a position to form a hydrogen bond with PI3K D238, but this residue has no specific interaction with RalGDS. This may explain the sensitivity of PI3K to the Ras Y64G mutation.

The PI3K γ C2 domain (residues 357-522) is an eight-stranded antiparallel β -sandwich consisting of two four-stranded β -sheets (Fig. 6). The fold of this domain is the same as the type II C2 domain found in PLC δ 1¹⁸. The N-terminal regions of all three PI3K classes have C2 domains, while the class II enzymes have an additional C2 domain at the C-terminus (Fig. 1). The segments leading from the RBD into the C2 domain and from the C2 domain to the helical domain are not ordered.

C2 domains are often involved in Ca²⁺-dependent or Ca²⁺-independent phospholipid membrane binding using three loops known as CBRs located at one end of the domain. The CBRs for PI3K γ are the loops connecting β 1 with β 2 (CBR1), β 3 with β 4 (CBR2), and β 5 with β 6 (CBR3). The CBR3 of PI3K γ is quite long compared to other C2 domains and is disordered in our structure. The C2 domain interacts primarily with the helical domain, but it also interacts with the linker segment before the RBD and with the C-terminal lobe of the catalytic domain. The surface of the C2 domain contacting the rest of PI3K γ is nearly identical to the surface of the PLC δ 1 C2 domain that contacts the catalytic domain of PLC δ 1.

PI3K can bind phospholipid membranes in the absence of other protein components, in a Ca²⁺-independent manner and carry out processive catalysis at the

membrane surface. By analogy with other enzymes such as protein kinase C and cytosolic phospholipase A2, it may be that the C2 domain of PI3K participates in membrane interaction. Consistent with this, we have found that the isolated C2 domain from PI3K γ binds multilamellar phospholipid vesicles similarly to the full-length enzyme (data not shown). In PI3K β and PI3K δ , CBR3 (residues 395-417 of PI3K δ) is particularly rich in basic residues that may be important for membrane binding.

The structure of a type II β phosphatidylinositol phosphate kinase (PIPK) was recently reported (19). This dimeric enzyme, which phosphorylates phosphoinositides at the 4-hydroxyl, consists of a single, catalytic domain. The dimer has an extensive flat, positively-charged surface that was proposed to be the membrane-binding interface of the enzyme. Although the N-lobe of PIPK is structurally related to the catalytic domain of PI3K γ , the location of the PI3K γ C2 domain with respect to the catalytic domain would sterically preclude membrane interactions using the surface of PI3K γ analogous to the putative PIPK membrane-binding surface. Given the location of the membrane-binding loops from the C2 domain and the cavity in the catalytic domain that must accommodate the PtdIns(4,5)P₂ headgroup, the membrane-binding surface of PI3K γ would be such that the CBRs, the crevice between the N- and C-lobes of the catalytic domain and the tip of the activation loop would face the membrane interface (Fig. 4A, right panel would represent a view from the membrane surface).

The helical domain of PI3K (residues 545-725) consists of five A/B pairs of anti-parallel helices (Fig 7). The first two pairs have one kinked helix each, hB1/hB1' and hB2/hB2'. This region has been variously referred to as HR2, the PI3K accessory